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A Leukocyte Type of 12-Lipoxygenase Is Expressed in Human Vascular and Mononuclear Cells

Evidence for Upregulation by Angiotensin II

Jeong A. Kim, Jia-Li Gu, Rama Natarajan, Judith A. Berliner, Jerry L. Nadler

Abstract The lipoxygenase (LO) pathway has been implicated in leading to accelerated atherosclerosis. However, the precise type of LO present in unstimulated human aortic smooth muscle cells (HSMC), endothelial cells (HAEC), and monocytes (MO) is not clear. In this study, we used a specific reverse-transcriptase polymerase chain reaction (RT-PCR) method to analyze the type of LO mRNA expressed in normal HSMC, HAEC, and MO. In all three cell types, a 333-base-pair band was seen when primers and probes specific for the leukocyte type of 12-LO were used, suggesting that a leukocyte type of 12-LO is expressed in these cell types. Western immunoblotting analysis in cultured HSMC, HAEC, and MO using a polyclonal peptide antibody to the leukocyte type of 12-LO showed a specific 72-kD band that is identical to the molecular weight of the leukocyte type of 12-LO. These results indicate that a leukocyte type of 12-LO RNA and protein are expressed in HSMC, HAEC, and MO. Further, angiotensin II upregulates 12-LO activity and expression in HSMC, supporting a role for this 12-LO pathway in human vascular disease. ***(Arterioscler Thromb Vasc Biol.* 1995;15:942-948.)**

Key Words • vascular smooth muscle cells • endothelial cells • atherosclerosis • hydroxyeicosatetraenoic acids • hypertension

The three mammalian lipoxygenases (LO) are named according to the carbon position (5, 12, or 15) at which they oxygenate arachidonic acid.[1] There is increasing evidence that certain LO enzymes are involved in the pathogenesis and acceleration of atherosclerosis by inducing oxidation of LDL to its atherogenic form[2,3] and increasing the growth or migration of smooth muscle cells.[4,5] In addition, new evidence suggests that a 12-LO protein plays a role in mediating angiotensin II (Ang II)–induced vascular and adrenal actions.[6-8] Recent studies indicate that at least two forms of 12-LO exist: The human platelet type 12-LO cloned from human erythroleukemia cells has been found primarily in platelets.[9,10] The other, a porcine leukocyte-type 12-LO, has been isolated and cloned from porcine leukocyte cells,[11,12] porcine pituitary cells,[13] and bovine tracheal cells.[14,15] We recently demonstrated the presence of a leukocyte type of 12-LO in human adrenal glomerulosa cells.[16] Human 15-LO has been purified from human and rabbit reticulocytes.[17,18] The human platelet and porcine leukocyte-type 12-LO share 65% amino acid homology.[9] However, porcine leukocyte-type 12-LO is highly homologous to the human 15-LO (86%).[12] Recently, it has been shown that 15-LO is expressed in macrophages of human atherosclerotic lesions but not in unstimulated monocytes (MO).[19-21]

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From the City of Hope National Medical Center and UCLA School of Medicine (J.A.B.), Los Angeles, Calif.

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Correspondence to Jerry L. Nadler, MD, City of Hope Medical Center, 1500 E Duarte Rd, Duarte, CA 91010.

In the present study, we evaluated the precise type of LO present in unstimulated human aortic smooth muscle cells (HSMC), endothelial cells (HAEC), and MO. Furthermore, since Ang II can increase the expression of 12-LO in human adrenal cells, we also evaluated the effects of Ang II on 12-LO regulation in HSMC. The results show that a 12-LO similar to that found in human adrenal glomerulosa is expressed in the normal HSMC, HAEC, and MO. Furthermore, this 12-LO is markedly upregulated by Ang II in HSMC.

Methods

Cells and Cultures

HAEC and HSMC were isolated from aortic specimens obtained from the heart donors in the UCLA heart transplant program. HAEC at passages 5 through 9 and HSMC at passages 3 through 7 were used. HAEC were grown in medium 199 containing 20% fetal bovine serum (FBS) supplemented with endothelial cell growth supplement (20 mg/mL) and heparin (90 μg/mL). HAEC were identified by their typical cobblestone morphology, presence of factor VIII–related antigen, and uptake of acetylated LDL labeled with 1,1′-dioctadecyl-1-3,3,3′3′-tetramethylindo-carbocyanine perchlorate (Dil-acetyl-LDL).[22] HSMC were grown in medium 199 containing 20% FBS and identified morphologically and immunohistochemically by use of HHF35, which was then visualized by a fluorescently labeled second antibody or with a biotin-streptavidin complex immunoperoxidase system.[23] MO were obtained from a large pool of healthy donors by a modification of the Recalde method.[24] These cells were approximately 73±8% MO.

The MO were suspended at 15×10^6 cells/mL in RPMI 1640 medium supplemented with L-glutamine, penicillin, and streptomycin. Cells were allowed to adhere to 100-mm polystyrene tissue culture plates for 3 hours at 37°C in the presence of 5% CO_2. The nonadherent cells were removed by rinsing the plates three times with PBS. The adherent cells were incubated in

Primers and Probes for Amplification and Detection

		Sequence (5'-3')	Position
Human 15-LO	5' Primer	AACTCAAGGTGGAACTACCGGAG	146-168
	3' Primer	ATATAGTITGGCCCCAGCCATATTC	453-477
	Probe	AGGCTCAGGACGCCGTTGCC	306-326
Leukocyte 12-LO	5' Primer	TTCAGTGTAGACGTGTCGGAG	145-165
	3' Primer	ATGTATGCCGGTGCTGGCTATATTT	451-477
	Probe	TCAGGATGCGGTCGCCCTCCAC	301-322
Human GAPDH	5' Primer	CCCATCACCATCTTCCAGGAG	211-231
	3' Primer	GTTCTCATGGATGACCTTGGC	475-495
	Probe	CTAAGCAGTTGGTGGTGCAGG	446-466
Human platelet 12-LO	5' Primer	GATGATCTACCTCCAAATATG	472-492
	3' Primer	CTGGCCCCAGAAGATCTGATC	610-630
	Probe	GTTTGAGGGCCATCTCCAGAGC	544-565

LO indicates lipoxygenase.

10% FBS containing RPMI medium for 36 hours in the presence or absence of interleukin (IL)-4 (400 pmol/L, R&D Systems).[21-25]

HSMC and HAEC monolayers were washed twice with ice-cold PBS and then processed for RNA extraction or Western analysis as described below. For HETE assay, approximately 24 hours prior to an experiment, the medium was replaced with medium 199 containing 0.4% FBS and 0.2% BSA.

cDNAs

Recombinant Bluescript plasmid containing the cDNA for human reticulocyte 15-LO was kindly provided by Dr E. Sigal (Syntex Co). pUC19 plasmid containing the cDNA for porcine leukocyte 12-LO was kindly provided by Drs S. Yamamoto and T. Yoshimoto (Tokushima University, Tokushima, Japan).[12] Bluescript plasmid containing the cDNA for human platelet 12-LO was kindly provided by Prof Bengt Samuelsson (Karolinska Institute, Stockholm, Sweden).[10]

Oligonucleotide Primers and Probes for Polymerase Chain Reaction

β_2-Microglobulin oligonucleotides were a kind gift of Dr Perrin White (Cornell University Medical College, New York, NY). Other oligonucleotides, including human GAPDH oligonucleotides, were synthesized on an Applied Biosystems DNA synthesizer and were purified by polyacrylamide gel electrophoresis. The sequences of oligonucleotides are listed in the Table; they were designed on the basis of known gene sequences[10,12,26,27] and selected from regions displaying the greatest divergence between porcine 12-LO and 15-LO sequences.[9]

Amplification of Reverse-Transcribed RNA by the Polymerase Chain Reaction

Total RNA from cultured HSMC and HAEC and freshly isolated MO was extracted with guanidiumthiocyanate-phenol-chloroform with RNAzol (Cinna/Biotecx Laboratories International Inc) or RNA stat-60 (Tel-test B, Inc). Some RNA samples were treated with RNAse-free DNAse. Total RNA (2.5 to 3 μg) was mixed with the polymerase chain reaction (PCR) buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 0.001% gelatin), 200 μmol/L of each of the four deoxynucleotide triphosphates, 25 pmol each of 5'- and 3'-primers, 2 U avian myeloblastosis virus reverse transcriptase (20 U/μL; Life Sciences), and 2.5 U *Taq* polymerase (Perkin Elmer Cetus) in a final volume of 50 μL. In some reactions, 5 pmol each of 5'- and 3'-primer of β_2-microglobulin or GAPDH was added as an internal standard. The samples were placed in a thermal cycler at 37°C for 8 minutes for the reverse transcription (RT) reaction to proceed. Then, conditions used for PCR were a denaturation step at 94°C for 1 minute, annealing at 50°C for 2 minutes, and extension at 72°C for 2 minutes for 20 to 40 cycles. Blank reactions with no RNA template were carried out through the RT and PCR steps. The human 15-LO cDNA, porcine leukocyte 12-LO cDNA, and human platelet 12-LO cDNA amplifications were carried out by mixing 2 to 5 ng cDNA plasmid in a 50-μL volume containing 200 μmol/L of each of the four deoxynucleotide trisphosphates, 25 pmol 5'- and 3'-primers, and 2.5 U *Taq* polymerase. The conditions for semiquantitative PCR were the same as described before.[16]

Gel Analysis and Blot Hybridization

Aliquots (20 μL) of the PCR products were subjected to electrophoresis in a 1.8% agarose gel in Tris acetate–EDTA buffer. After being stained with ethidium bromide and photographed, the gel was transferred onto a Zeta-probe membrane (Bio-Rad) by capillary blotting. The oligonucleotides used as probes were labeled at the 5'-end with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs) and hybridized with membrane overnight in 6×SSC (1×SSC contains 0.15 mol/L NaCl/0.015 mol/L sodium citrate), 0.5% nonfat dried milk, and 7% SDS at 42°C. Membranes were washed once in 6×SSC at room temperature for 15 minutes and then once at 60°C for 15 minutes. The washing conditions were worked out to distinguish the PCR products of human 15-LO from those of porcine leukocyte 12-LO.[16] The filters were exposed to Kodak x-ray film (Eastman Kodak Co) with an intensifying screen at −70°C. Blots were quantified with a computerized video densitometer.

Western Immunoblotting

Cell pellets were lysed in lysis buffer containing PBS (pH 7.3), 1% Triton X-100, 1 mmol/L PMSF, 50 μmol/L leupeptin, and 0.1% SDS. Lysates were centrifuged at 10 000*g* for 10 minutes. An aliquot of the supernatant (cytosol) was saved for protein estimation, and the remainder was saved at −70°C for Western blot analysis.

SDS polyacrylamide gel electrophoresis (10% running gel, 4% stacking gel) was performed according to the method of Laemmli.[28] For Western blotting, gels were equilibrated in transfer buffer (35 mmol/L Tris base, 192 mmol/L glycine, and 20% methanol, pH 8.3) and then transferred to nitrocellulose (Hybond, Amersham), as described by Towbin et al,[29] in a semidry polyblot apparatus (American Bionetics, Inc) for 40 minutes. The nonspecific sites were blocked with PBS containing 10% fetal calf serum (FCS) at 4°C overnight. The membranes were then washed twice with PBST (PBS + 0.05% Tween-20) and incubated with primary antibody in PBST containing 1% BSA and 20% (vol/vol) FCS for 2 hours at room temperature. A polyclonal antibody against porcine 12-LO peptide with the sequence of amino acids 646 through 662 of the porcine leukocyte 12-LO sequence[12] was used. This antiserum was used at 1:100 dilution. In some studies, a polyclonal antibody against human 15-LO kindly provided by Dr E. Sigal

(Syntex Co) was used. The washed membranes were then incubated for 1 hour with second antibody (goat anti-rabbit) conjugated with alkaline phosphatase (1:5000; Promega). Detection was either by color development using substrate mixture (nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, Promega) or by chemiluminescence using CSPD substrate and the Western-Light Chemiluminescent detection system (Tropix, Inc). Nonspecific binding was evaluated with normal rabbit serum. Western blots were quantified with a computerized video densitometer (Applied Imaging; Lynx DNA Vision), and values were expressed as arbitrary absorbance units.

Measurement of 12-LO Products

These assays were performed according to previously published methods.[6,7] Briefly, 12- and 15-HETE are extracted from supernatants and cells on C18 mini columns (Analytichem International) and measured by our validated reverse-phase gradient high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA) methods. The HPLC system provides a 0.7-minute separation time between 12- and 15-HETE peaks. The 12-LO antibody used for RIA recognizes only 12-*S*-HETE, with less than 0.1% cross-reactivity with 12-*R*-HETE and 0.3% with 15-HETE.

Measurement of LO Activity in HSMC

Confluent HSMC were placed in medium plus 10% FCS 24 hours prior to the experiment. The cells were harvested, washed, suspended in 1 mL Tris-HCl buffer (25 mmol/L, pH 7.7), and then sonicated on ice. The assay mixture contained, in 1.0 mL, 800 μL enzyme (sonicate), 100 μL $CaCl_2$ (1.5 mmol/L), and 50 μL glutathione (0.5 mmol/L). An enzyme blank was run simultaneously. The reaction was started at 37°C with 50 μL sodium arachidonate (160 μmol/L Nu Check Prep) or 0.25 μCi [^{14}C]linoleic acid (New England Nuclear). After 10 minutes of incubation, the reaction was stopped with 2 mL isopropanol/1.2% acetic acid followed by 2 mL chloroform. The lower organic layer was filtered and subjected to HPLC to detect HETEs using our gradient reverse-phase HPLC system.[6,7] A 12-HETE peak was identified by UV detection at 237 nm and comigration with authentic standard (retention time, 18.3 minutes). Peak heights were quantified with a Shimazu CR5A integrator.

Data Analysis

Immunoblots and autoradiograms were analyzed with a computer-driven densitometer (Applied Imaging; Lynx DNA Vision). Data shown are representative of 2 or 3 experiments. Data generated from Ang II treatment of HSMC for 12-HETE synthesis were analyzed by ANOVA for multiple samples using a statistical package on a Macintosh computer system. Data are presented as mean±SEM.

Results

Expression of a Leukocyte Type of 12-LO mRNA in HAEC, HSMC, and MO

The expression of 12-LO mRNA in HAEC, HSMC, and MO was evaluated by a specific semiquantitative RT-PCR method, since the level of detection was below the sensitivity of Northern analysis. Fig 1A shows expression of leukocyte 12-LO mRNA in normal HAEC, HSMC, and MO by a method highly specific for this form of 12-LO mRNA. The appropriate 333-base-pair (bp) band was seen in all three cell types.

Fig 1B demonstrates RT-PCR analysis of human 15-LO mRNA expression from the same RNA. These results reveal no evidence for a band characteristic of human 15-LO. In a separate experiment, RNA from HAEC, HSMC, and MO was amplified and probed for the platelet-type 12-LO RNA. No evidence for a human platelet 12-LO expression was found (data not shown).

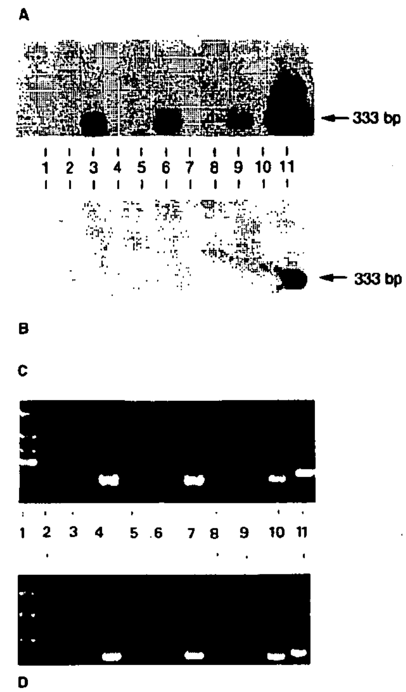

FIG 1. Reverse transcriptase–polymerase chain reaction analysis of leukocyte 12-lipoxygenase (LO) RNA in human aortic endothelial cells (HAEC), human aortic smooth muscle cells (HSMC), and monocytes (MO). A, RNA samples were amplified for 40 cycles with porcine leukocyte-specific 12-LO primers. Membranes were hybridized with porcine leukocyte 12-LO oligonucleotide probe. Lane 1 is a marker; lanes 2, 5, and 8 are negative controls without template; lane 3 represents total RNA from HAEC, with porcine leukocyte 12-LO primer and lane 4 with GAPDH primers; lane 6 represents total RNA from HSMC with porcine leukocyte 12-LO primers and lane 7 with GAPDH primers; lane 9 represents total RNA from MO with porcine leukocyte 12-LO primers and lane 10 with GAPDH primers; and lane 11 is a positive control using the porcine leukocyte 12-LO cDNA. B, Same RNA samples were amplified for 40 cycles with human specific 15-LO primers. Membranes were hybridized with human 15-LO oligonucleotide probe. Only the 333-base-pair (bp) product from amplification of the 15-LO cDNA (positive control) is shown. C, Ethidium bromide–stained agarose gel of A. D, Ethidium bromide–stained agarose gel of B.

Evidence for Selective Increase in 15-LO mRNA in MO Exposed to IL-4

Fig 2A shows expression of human 15-LO mRNA in MO by IL-4 exposure for 36 hours. However, no 15-LO mRNA was seen in fresh or cultured human MO in the absence of IL-4.

Fig 2B demonstrates expression of leukocyte 12-LO mRNA in the same RNA samples as in Fig 2A. Interestingly, 12-LO mRNA expression was actually reduced with IL-4 treatment. The left panels are ethidium bromide–stained gels, and the right panels are autoradiograms.

Expression of a Leukocyte Type of 12-LO Protein in HAEC, HSMC, and MO

To investigate whether a leukocyte type of 12-LO enzyme was expressed in HAEC, HSMC, and circulating

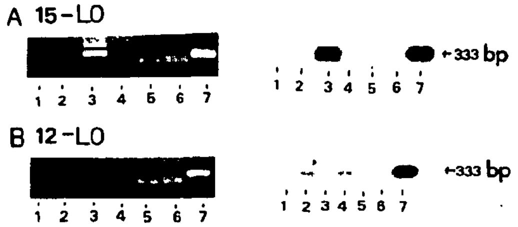

FIG 2. Increase in human 15-lipoxygenase (LO) RNA in monocytes (MO) exposed to interleukin (IL)-4. A, RNA samples were amplified for 23 cycles with GAPDH primers or 40 cycles with human 15-LO primers. Membranes were hybridized with human 15-LO oligonucleotide probe. Lane 1 is a negative control without template; lane 2 represents total RNA from MO incubated in 10% fetal bovine serum containing RPMI 1640 for 36 hours; lane 3 from MO treated with IL-4 (400 pmol/L) for 36 hours; and lane 4 from freshly isolated MO with human 15-LO primers. Lanes 5 and 6 represent same RNA samples as lane 2 and lane 3, respectively, with GAPDH primers. Lane 7 is a positive control using the human 15-LO cDNA. Ethidium bromide–stained agarose gel is shown in the left panel. B, Same RNA samples were amplified with leukocyte 12-LO primers. Membranes were hybridized with leukocyte 12-LO oligonucleotide probe. Lane 7 is a positive control using the leukocyte 12-LO cDNA. Ethidium bromide–stained gel is shown in the left panel.

MO, the 10 000*g* supernatant proteins were electrophoresed and subjected to Western analysis using a polyclonal peptide antibody derived from a sequence in the porcine leukocyte type of 12-LO that is homologous to the sequence of 12-LO found in human adrenal glomerulosa. This antibody has previously been shown to lack cross-reactivity to the platelet form of 12-LO and successfully demonstrated the presence of a leukocyte-type 12-LO in human adrenal cells.[16] Fig 3 demonstrates a major 72-kD band from Western analysis in HSMC, HAEC, and MO. Western analysis performed similarly using a polyclonal antibody directed against the human 15-LO protein did not demonstrate a band in the expected molecular weight from these cells (data not shown). HAEC and MO produced 12-*S*-HETE as reflected by HPLC and RIA analysis (HAEC 2386, MO 820 pg/10^6 cells). Results for HSMC are detailed below.

Therefore, HSMC, HAEC, and MO appear to express a 12-LO protein similar to the leukocyte type of 12-LO found in porcine tissues and human adrenal glomerulosa.

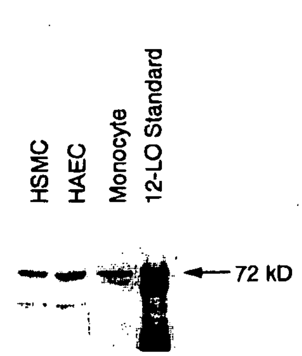

FIG 3. Expression of leukocyte 12-lipoxygenase (LO) protein (72 kD) in normal human aortic endothelial cells (HAEC), human aortic smooth muscle cells (HSMC), and monocytes (MO). Cytosol fractions from HAEC, HSMC, and MO were electrophoresed along with authentic porcine 12-LO protein and subjected to Western immunoblotting as described under "Methods."

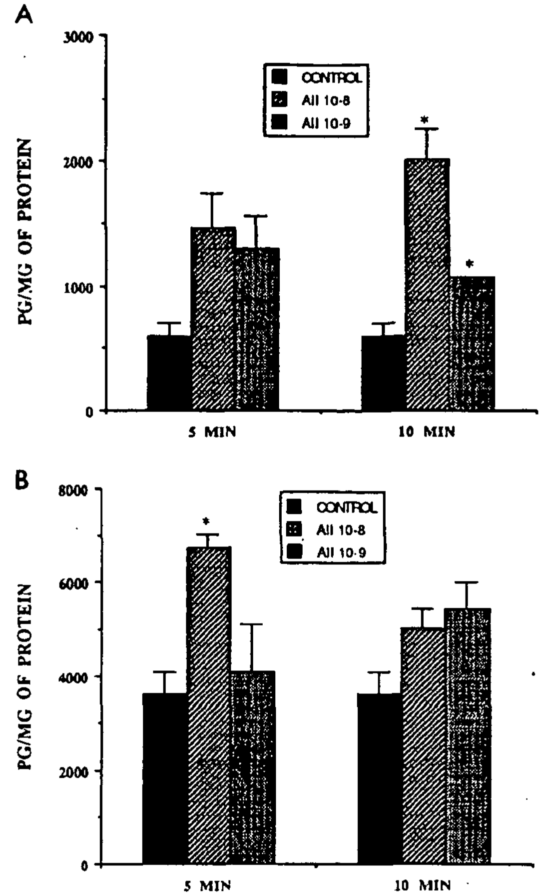

FIG 4. Bar graphs. A, Effect of angiotensin II (Ang II) on 12-HETE release by human aortic smooth muscle cells (HSMC). HSMC were grown to confluency. Serum was removed, and cells were incubated in medium 199 containing 0.4% fetal bovine serum (FBS) and 0.2% BSA for 18 hours. Cells were then washed with Dulbecco's modified Eagle's medium (DMEM) and incubated for 20 minutes in DMEM containing 0.2% BSA. Ang II was added to the cells for 5 and 10 minutes at concentrations of 10^{-9} and 10^{-8} mol/L. Media were collected for HETE assay by high-performance liquid chromatography and radioimmunoassay. * $P<.05$ vs control, n=4. B, Effect of Ang II on cell-associated 12-HETE levels in HSMC. After supernatants were collected, cells were washed with ice-cold PBS and harvested by scraping for the assay of cell-associated HETEs as described in the "Methods" section. * $P<.02$ vs control, n=4.

Effect of Ang II on 12-LO Activity and Expression in HSMC

Fig 4A shows that 5 minutes of incubation of HSMC with Ang II at the concentrations of 10^{-8} and 10^{-9} mol/L in serum-free medium stimulates the release of 12-HETE (control, 599±105; Ang II 10^{-8} mol/L, 1467±277; Ang II 10^{-9} mol/L, 1296±262 pg/mg protein). Ten-minute incubations with Ang II significantly stimulated the release of 12-HETE at the concentration of 10^{-8} mol/L. Ang II also significantly increased cell-associated 12-HETE levels in HSMC (Fig 4B). In other studies, it was found that 12-HETE release in the medium in response to Ang II (10^{-7} mol/L) could be reduced by the LO inhibitor cinnamyl-3,4-dihydroxy-α-cyanocinnamate (CDC, 10^{-5} mol/L) (control, 1064±60; Ang II, 3297±178; Ang II+CDC, 1862±116 pg/mg protein, $P<.0001$ versus Ang II alone). Another structurally distinct 12-LO inhibi-

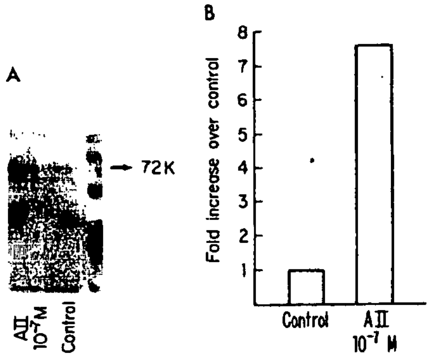

Fig 5. Regulation of leukocyte 12-lipoxygenase protein expression by angiotensin II (Ang II, AII in figure) in human aortic smooth muscle cells. A, Immunoblot showing regulation by Ang II. B, Bar graph representation of densitometric analysis of immunoblot in A. Cells were grown in medium 199 containing 20% fetal bovine serum (FBS) and were serum-depleted for 24 hours by placement in medium 199 containing 0.4% FBS and 0.2% bovine serum albumin. Cells were treated with Ang II at the concentration of 2×10^{-7} mol/L for 24 or 48 hours. Cells were washed with PBS and harvested by scraping. Cell pellets were lysed and cytosol fractions were electrophoresed as described under "Methods."

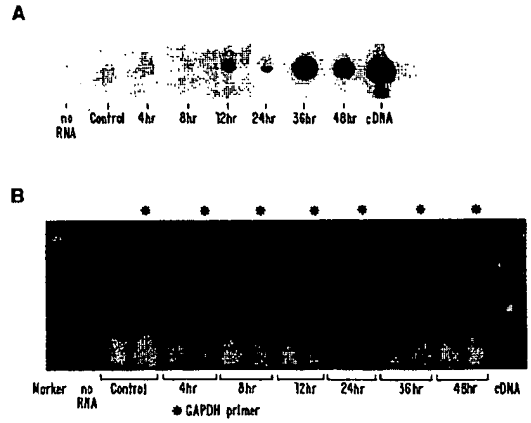

Fig 6. Regulation of leukocyte 12-lipoxygenase (LO) mRNA levels by angiotensin II (Ang II) in human aortic smooth muscle cells (HSMC) by reverse transcriptase–polymerase chain reaction. A, Autoradiogram of the blot hybridized with porcine leukocyte-type 12-LO oligonucleotide probe. B, Ethidium bromide–stained agarose gel. Total RNA was extracted from cultured HSMC incubated in low-serum conditions with Ang II (2×10^{-7} mol/L) for the different time periods shown. RNA samples were amplified for 40 cycles with leukocyte 12-LO primers or GAPDH primers.

tor, baicalein (10^{-5} mol/L), also reduced the cell-associated increases in 12-HETE in response to Ang II (control, 9.9±0.66; Ang II, 20.6±2.46; Ang II+baicalein, 15.7±1.2 ng/mg protein, $P<.01$ versus Ang II alone).

To examine whether Ang II induces the 12-LO enzyme expression in HSMC, cells were treated with Ang II at a concentration of 2×10^{-7} mol/L for 24 or 48 hours. The 12-LO protein was identified by Western immunoblotting using a specific antibody to purified leukocyte-type 12-LO or a peptide antibody derived from known sequences present in the human leukocyte type of 12-LO. A distinct band was detected with a molecular weight of nearly 72 kD, which is the reported molecular weight of the porcine leukocyte type of 12-LO (Fig 5). A 24-hour incubation of HSMC with Ang II in serum-free medium induced nearly a sevenfold increase in 12-LO protein expression (Fig 5). In other experiments, Ang II added for 48 hours also increased 12-LO expression fourfold to sevenfold (data not shown).

To evaluate the specific expression and regulation of 12-LO mRNA in HSMC, we used an RT-PCR assay that exclusively amplifies the leukocyte type of 12-LO. The size of the PCR-amplified fragment is 333 bp for both 12- and 15-LO. Therefore, specific conditions were used to distinguish leukocyte-type 12-LO and human 15-LO by increasing stringency and raising washing temperature to 60°C. Fig 6A shows a Southern blot analysis of RT-PCR–amplified products from HSMC that were serum-deprived for 24 hours and then treated for the indicated times with Ang II 10^{-7} mol/L. In this experiment, very low basal expression of 12-LO is seen. However, in other experiments in cells from various other donors, basal 12-LO expression is detectable with PCR at 20 to 30 cycles. Ang II induces 12-LO mRNA expression starting at the 12-hour incubation time, and the maximum induction is shown at 36 hours of incubation of cells with Ang II. Fig 6B shows the ethidium bromide–stained agarose gel showing the amplification of GAPDH as an internal marker. When PCR conditions were used that were specific for either the platelet-type 12-LO or human 15-LO, no specific RNA band was detected (data not shown). Therefore, basal serum-deprived HSMC show low expression of a leukocyte-type 12-LO, which is markedly upregulated by Ang II.

Discussion

The present results demonstrate for the first time that a 12-LO RNA and protein similar to that found in porcine leukocytes and human adrenal glomerulosa[12,16] are also expressed in human vascular cells and circulating MO. Several approaches were used in this present investigation to support this conclusion. First, a peptide antibody derived from a sequence common to the porcine and human forms of leukocyte-type 12-LO revealed a characteristic 72-kD band in HSMC, HAEC, and MO lysates. This antibody does not cross-react with the platelet form of 12-LO but has partial cross-reactivity with human 15-LO.[16] Second, a highly specific RT-PCR procedure was used to detect 12-LO mRNA in these cell types. A previous study demonstrated, using this technique, that a leukocyte type of 12-LO was the exclusive type of 12-LO seen in human adrenal glomerulosa and U937 cells.[16] In the present study, a specific 333-bp amplified mRNA product was found in unstimulated HSMC, HAEC, and MO when appropriate leukocyte-type 12-LO primers and probe were used. Third, in all three cell types, the 12-LO product 12-*S*-HETE was formed, as reflected by HPLC and specific RIA, which recognizes only the *S* enantiomer of 12-HETE. The cytosol from HSMC reacted with LO to produce 12-HETE or linoleic acid to form 13-hydroxyoctadeca-9*Z*,11*E*-dienoic acid (data not shown). This reaction is characteristic of a leukocyte type of 12-LO and not the platelet 12-LO, which reacts only with arachidonic acid to produce 12-HETE.

The human 15-LO originally cloned from the reticulocyte and found in human trachea is highly homologous (86% sequence homology) to the porcine leukocyte type

of 12-LO.[12] The PCR technique used here can distinguish between the leukocyte 12-LO and the 15-LO.[16] The specificity of this approach was demonstrated using the 12-LO and 15-LO cDNA as templates for amplification.[16] Therefore, the Southern blot hybridization using the leukocyte 12-LO probe provides the strongest evidence that the band seen reflects a 12-LO and not a 15-LO amplified product. These results are in agreement with previous studies showing no detectable 15-LO mRNA in basal or stimulated human endothelial or nonstimulated mononuclear cells.[30] However, 15-LO mRNA and protein have been found in macrophage-rich areas of atherosclerotic vascular lesions[19,20] and in IL-4 stimulated MO,[21-25] suggesting that 15-LO can play a role in advanced atherosclerotic and immune-mediated vascular disease. In the present report, we confirm the results of others[21-25] that IL-4 can increase 15-LO mRNA expression in human MO. In contrast, the 12-LO expression in these MO is actually reduced by IL-4, suggesting that different factors can regulate human 15-LO and the leukocyte type of 12-LO.

Another major finding of the present study is that Ang II increases the activity and expression of 12-LO mRNA and protein in HSMC. Increasing evidence suggests that a 12-LO enzyme plays an important role in Ang II–induced actions in several tissues. Studies suggest that the 12-LO pathway of arachidonic acid can mediate Ang II–induced aldosterone synthesis in rat and human adrenal glomerulosa cells.[6,7] Furthermore, recent data indicate that Ang II–induced adrenal cell proliferation is mediated at least in part by activation of a 12-LO enzyme.[8] Additional studies in the rat have implicated the 12-LO pathway in the vasoconstrictive and renin-inhibitory actions of Ang II.[31,32] The aorta has the capacity to produce LO products, including 12- and 15-HETE.[33] Recent data have revealed that both Ang II and high glucose can upregulate the leukocyte type of 12-LO activity and expression in cultured porcine aortic smooth muscle cells.[34,35]

Additional studies will be needed to fully evaluate the potential implication of the increased 12-LO activity and expression by Ang II in human vessel wall. Ang II has major effects on vascular smooth muscle cell growth in vitro and in vivo.[4,36-39] A recent report found that a relatively selective 12-LO inhibitor could completely prevent Ang II–induced hypertrophic responses in cultured porcine vascular smooth muscle cells.[4] Furthermore, 12-HETE induced increases in protein and fibronectin content of these vascular smooth muscle cells similar to those induced by Ang II.[4] The 12-LO pathway and its product, 12-HETE, have also been implicated in vascular smooth muscle cell migration.[5] 12-HETE at concentrations as low as 10^{-12} mol/L have been shown to lead to smooth muscle cell migration. Additional studies have demonstrated that 12-LO products can activate specific isoforms of protein kinase C and oncogenes, including *ras*, c-*fos*, and *jun*.[40-42] Therefore, increased 12-LO activity and expression by Ang II may be a previously unrecognized mechanism for Ang II–induced hypertensive and atherosclerotic vascular disease in humans, and blockade of the 12-LO pathway may be a novel therapeutic modality to reduce Ang II–related cardiovascular disease.

The 12-LO pathway in the human vascular wall and MO may participate in other mechanisms related to the development or progression of atherosclerotic vascular disease. Recent evidence has implicated an LO pathway in oxidative modification of LDL in the vascular wall.[19,20] It is now clear that HAEC, HSMC, or MO have the capacity to convert native LDL to minimally modified LDL, which has a greater atherosclerotic potential. Of interest are the data showing that cholesterol loading of macrophages leads primarily to increased production of 12-HETE.[43] A recent report has now demonstrated that both the leukocyte type of 12-LO and 15-LO can similarly oxidize lipoproteins.[44] Interestingly, this same report showed a lack of ability of the platelet 12-LO to oxidize lipoproteins.

The precise role of this 12-LO pathway in hypertensive and atherosclerotic disease in humans will require further study using specific methods to selectively inhibit this form of 12-LO. Currently, few data exist on appropriate pharmacological inhibitors that are selective for the leukocyte type of 12-LO. However, use of antisense or ribozyme methods to reduce leukocyte-type 12-LO activity should provide more definitive information as to the role of this newly defined pathway that may be relevant to human vascular disease.

Acknowledgments

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 NEWS 43   Jun 06  PASCAL enhanced with additional data

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L2               QUE  ABB=ON  PLU=ON  12S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L3               QUE  ABB=ON  PLU=ON  12(W)S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L4               QUE  ABB=ON  PLU=ON  12(W)HETE
L5               QUE  ABB=ON  PLU=ON  12S(W)HETE
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L12   3169683 DIAGNOS? OR DETECT? OR TEST? OR ASSAY? OR MARKER OR SCREEN?

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L13         6 L11 AND L12

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L13  ANSWER 1 OF 6  CA  COPYRIGHT 2003 ACS
AN   134:233363  CA
TI   Second human leukotriene B4 receptor, BLT2, and use in therapy and drug
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IN   Kato, Kazuhiko; Asai, Kenji; Nagaso, Hiroshi; Yokomizo, Takehiko; Shimizu,
     Takao
PA   Meiji Seika Kaisha, Ltd., Japan; Japan Health Sciences Foundation
SO   PCT Int. Appl., 52 pp.
     CODEN: PIXXD2
DT   Patent
LA   Japanese
FAN.CNT 1
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         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
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PRAI JP 1999-257525       A    19990910
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AB A novel human leukotriene B4 receptor, cDNA, recombinant expression, and methods and reagent kits for **screening** agonists or antagonists, are disclosed. A method for treatment or prevention of diseases assocd. with LTB4, hydroxyeicosatetraenoic acids (HETE), or hydroxyperoxyeicosatetraenoic acids (HPETE), is claimed. Antibodies to the receptor is claimed. Leukotriene B4 (LTB4) is a potent chemoattractant and activator of both granulocytes and macrophages. The actions of LTB4 appear to be mediated by a specific G protein-coupled receptor (GPCR) BLT1, originally termed BLT. Here, we report the mol. cloning of a novel GPCR for LTB4, designated BLT2, which binds LTB4 with a Kd value of 23 nM compared with 1.1 nM for BLT1, but still efficiently transduces intracellular signaling. BLT2 is highly homologous to BLT1, with an amino acid identity of 45.2%, and its open reading frame is located in the promoter region of the BLT1 gene. BLT2 is expressed ubiquitously, in contrast to BLT1, which is expressed predominantly in leukocytes. Chinese hamster ovary CHO-K1 cells expressing BLT2 exhibit LTB4-induced chemotaxis, calcium mobilization, and pertussis toxin-insensitive inhibition of adenylyl cyclase. Several BLT1 antagonists, including U 75302, failed to inhibit LTB4 binding to BLT2. Binding of [3H] LTB4 to BLT2 was inhibited by LTB4, 20-OH-LTB4, 12-epi-LTB4, 12(R)-HETE, **12(S)-HETE**, 15(S)-HETE, or 15(S)-HPETE, indicating binding of those compds. Thus, BLT2 is a pharmacol. distinct receptor for LTB4, and may mediate cellular functions in tissues other than leukocytes. **Screening** of agonist/antagonist was demonstrated with HEK-293 cells transformed with a plasmid construct contg. luciferase gene (luc) placed down stream of a cAMP response element (CRE). When cells were treated with FSK and LTB4, luciferase expression was elevated relative to the control (w/o LTB4).

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IN Pfenninger, Karl; De La Houssaye, Becky; Mikule, Keith; Helmke, Steve; Drabkin, Harry
PA University Technology Corporation, USA
SO PCT Int. Appl., 87 pp.
CODEN: PIXXD2
DT **Patent**
LA English
FAN.CNT 1

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MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9943109	A1	19991213	AU 1999-43109	19990521
US 6566047	B1	20030520	US 2000-463259	20000418

PRAI US 1998-86477P P 19980522
WO 1999-US11320 W 19990521

AB The present invention provides methods of identifying and using compds. that modulate cell motility. Such compds. can be used to inhibit cancer cell metastasis or promote neurite growth and regeneration. The methods generally relate to the repellent-receptor signaling pathway that controls cellular attachment and detachment to a substratum. The methods are accomplished by exposing whole cells or components of cells, such as pseudopods, to a putative repellent agent. The ability of the putative agent to activate a parameter of the repellent signaling pathway is then detd. The parameters **assayed** for activation in these methods include cytosolic phospholipase A2, 12-lipoxygenase, or protein kinase C.

RE.CNT 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 3 OF 6 CA COPYRIGHT 2003 ACS
AN 122:105410 CA
TI Preparation of caffeic acid amide derivatives as 12-lipoxygenase inhibitors
IN Matsuki, Shinsuke; Kiso, Yoshinobu; Cho, Hidetsura; Tamaoka, Mie; Murota, Seiitsu; Morita, Ikuo
PA Suntory Ltd, Japan
SO Jpn. Kokai Tokkyo Koho, 40 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 06247850	A2	19940906	JP 1993-57991	19930224
PRAI	JP 1993-57991		19930224		

OS MARPAT 122:105410
AB Caffeic acid amide derivs. [I; R1, R2 = H, COR4, C(S)R5, PO(OR6)OR7, or R1R2 forms a 5-membered ring; wherein R4 = C1-6 alkyl or alkoxy, C6-10 aryloxy, c712 aralkyloxy, substituted amino, cyclic amino; R6, R7 = C1-6 alkyl, C6-10 aryl, C7-12 aralkyl, alkali metal; R3 = OR1, OR2, H, OH, O2CR4, OC(S)R5, PO(OR6)OR7, wherein R1, R2, R4 - R7 = same as above; X, Y = H, (un)substituted C1-6 alkyl, C6-10 aryl, C7-12 aralkyl, C7-12 aralkyloxy, C7-12 arylalkenyl, C7-12 aryloxyalkenyl, heterocyclyl, or heterocyclylalkyl, or XY forms N-contg. heterocyclic ring; provided that both X = Y .noteq. H] and pharmacol. acceptable salts thereof, useful for the treatment of arteriosclerosis, ischemic heart diseases, etc., are prepd. A medicament for the treatment and prevention of diseases caused by unusual rise in the activity of 12-lipoxygenase, e.g. atrophy of brain blood vessel, allergy, inflammation, cancer metastasis, asthma, normal psoriasis, and nephritis, contains 12-lipoxygenase inhibitor or pharmacol. acceptable salts thereof as the active ingredient. Thus, a soln. of 2.40 g 3,4-dihydroxybenzaldehyde in DMF was added to a soln. of N-[2-(2-thienyl)ethyl]-2-cyanoacetamide in DMF and benzene followed by adding a few drops of piperidine and the resulting mixt. was refluxed for 1 h to give 86% I (R1 = R2 = R3 = X = H, Y = Q) (II). In 12-lipoxygenase inhibition **assay**, II and I (R1 = R2 = R3 = X = H, Y = Q1) at 10-6 M in vitro inhibited the prodn. of **12-HETE** in rat platelet rich plasma,by 77.2 and 80.1%, resp.

L13 ANSWER 4 OF 6 CA COPYRIGHT 2003 ACS
AN 114:185525 CA
TI Preparation of 1,4-thiazine derivatives as phospholipase A2, lipoxygenase,

and cyclooxygenase inhibitors
IN Naka, Tatsuhiko; Hisano, Masahiro
PA Takeda Chemical Industries, Ltd., Japan
SO Jpn. Kokai Tokkyo Koho, 23 pp.
CODEN: JKXXAF
DT **Patent**
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 02275869	A2	19901109	JP 1990-20843	19900130
	JP 2967231	B2	19991025		
PRAI	JP 1989-21921		19890130		

OS MARPAT 114:185525
AB 1,4-Thiazine derivs. [I; A = N, B = (substituted) pyrrolyl, NH2, alkylthio; A = NR2 wherein R2 = H, (substituted) aliph. hydrocarbyl; B = oxo, thioxo, (substituted) hydrazono, etc.; R1 = H, alkoxy, acyloxy, alkylthio, etc.; R3 = (substituted) aliph. or arom. hydrocarbyl, (esterified) CO2H, carbamoyl, etc.; R4 = H, aliph. or arom. hydrocarbyl; dotted line denotes satn. or unsatn.] are prepd. A soln. of PhCOCH2Br in MeCOEt was added dropwise to a suspension of HSCH2CONH2 and Et3N in MeCOEt under cooling and the mixt. refluxed to give 50% I (R1 = R4 = H, R3 = Ph, A = NH, B = oxo). I showed IC50 of 0.078-3.5 .mu.M against LTB4, 0.081-3.5 .mu.M against 5-HETE, 4.5-35.0 .mu.M against phospholipase A2, and antihypoxia activity in mice at 10 mg/kg i.p., etc. Also prepd. and **tested** were 143 addnl. I. Tablet, capsule, and ointment formulations were given.

L13 ANSWER 5 OF 6 CA COPYRIGHT 2003 ACS
AN 112:197871 CA
TI Preparation and formulation of caffeic acid derivatives as 12-lipoxygenase inhibitors
IN Cho, Hidetsura; Ueda, Masaru; Tamaoka, Mie; Hamaguchi, Mikiko; Murota, Seiitsu; Morita, Ikuo
PA Suntory, Ltd., Japan
SO Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DT **Patent**
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 339671	A2	19891102	EP 1989-107771	19890428
	EP 339671	A3	19910515		
	EP 339671	B1	19940810		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 01275552	A2	19891106	JP 1988-106274	19880428
	JP 2557090	B2	19961127		
	JP 02235852	A2	19900918	JP 1989-55867	19890308
	JP 2647185	B2	19970827		
	AU 8933813	A1	19891102	AU 1989-33813	19890428
	AU 618595	B2	19920102		
	US 5063243	A	19911105	US 1989-344583	19890428
PRAI	JP 1988-106274		19880428		
	JP 1989-55867		19890308		

OS MARPAT 112:197871
AB Caffeic acid derivs. [I; X = H, OH; R1 = H, straight or branched C1-20 alkyl or alkenyl, (CH2)nZR2; n = 1-10; Z = O, CH:CH, a single bond; R2 = (un)substituted Ph or heterocyclyl], useful for curing and preventing circulatory diseases, e.g. arteriosclerosis, are prepd. Thus, to a soln. of 3,4-dihydroxybenzaldehyde were added NCCH2CO2Et and piperidine and the resulting mixt. was kept 20 h at room temp. to give I (X = H, R1 = Et) (II). In 12-lipoxygenase inhibition **test**, II lowered the prodn. of **12-hydroxyeicosatetraenoic acid** (5-HETE)

by 38.1% in a dild. and citrated blood collected from the abdominal aorta of rat.

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L13  ANSWER 6 OF 6  CA  COPYRIGHT 2003 ACS
AN   109:73166  CA
TI   Preparation, testing, and formulation of alkadienoic and
     alkatrienoic anilide derivatives as 5-lipoxygenase inhibitors.
IN   Arai, Yoshinobu; Nakai, Hisao; Toda, Masaaki
PA   Ono Pharmaceutical Co., Ltd., Japan
SO   Jpn. Kokai Tokkyo Koho, 33 pp.
     CODEN: JKXXAF
DT   Patent
LA   Japanese
FAN.CNT 1
     PATENT NO.         KIND  DATE          APPLICATION NO.  DATE
     ---------------    ----  --------      ---------------  --------
PI   JP 62230760        A2    19871009      JP 1986-71033    19860331
PRAI JP 1986-71033            19860331
AB   The title compds. [I; A = bond, (C1-4 alkyl-substituted) vinylene,
     ethylene, ethynylene, Q1 wherein T = O, S; B = heterocyclyl; R1 =
     carboxyl, 5-tetrazolyl; R2 = H, C1-4 alkyl; R3 = H, halo, C1-4 alkyl;
     dotted line denotes cis-double or triple bond; n = 7,8], useful as
     5-lipoxygenase inhibitors, are prepd.  To 160 mg 8-(N-methylamino)-1,4-
     benzodioxane-2-carboxamide in a CH2Cl2-pyridine mixt. was added dropwise a
     mixt. of 214 mg(2E,11Z,14Z)-eicosa-2,11,14-trienoic acid with an excess
     (COCl)2 in CH2Cl2 to give 340 mg benzodioxane deriv. II (R1 = CONH2) which
     in DMF was added dropwise to POCl3 in DMF to give 295 mg nitrile II (R1 =
     cyano) (III).  III 295, NaN3 190, and NH4Cl 190 mg were mixed in DMF and
     heated at 80.degree. for 20 min and then 110.degree. for 20 min to give
     300 mg II (R1 = 5-tetrazolyl) the Na salt of which had an IC50 of 2.2
     .mu.M for inhibition of 5-HETE, 5,12-HETE, and
     leukotriene B.  One hundred tablets were prepd. by pelletizing a mixt. of
     I 5.0, cellulose Ca gluconate 0.2, Mg stearate 0.1, and microcryst.
     cellulose 4.7 g.


=> set range =(,13CI)
SET COMMAND COMPLETED

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     FILE 'CA' ENTERED AT 13:08:19 ON 12 JUN 2003
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L2              QUE  ABB=ON  PLU=ON  12S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L3              QUE  ABB=ON  PLU=ON  12(W)S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L4              QUE  ABB=ON  PLU=ON  12(W)HETE
L5              QUE  ABB=ON  PLU=ON  12S(W)HETE
L6              QUE  ABB=ON  PLU=ON  12(W)S(W)HETE
L7              QUE  ABB=ON  PLU=ON  12HETE
L8              QUE  ABB=ON  PLU=ON  12SHETE
L9              QUE  ABB=ON  PLU=ON  L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR
                ---------
L10         1475 S L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9
L11           20 S L10 AND P/DT
L12      3169683 S DIAGNOS? OR DETECT? OR TEST? OR ASSAY? OR MARKER OR SCREEN?
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L13            6 S L11 AND L12
                SET RANGE =(,13CI)

=> s l1 or l2 or l3 or l4 or l5 or l6 or l7 or l8 or l9
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2849710 ACID
1154220 ACIDS
3251175 ACID
          (ACID OR ACIDS)
    434 12(W)HYDROXYEICOSATETRAENOIC(W)ACID
   1185 12S
   1629 HYDROXYEICOSATETRAENOIC
2849710 ACID
1154220 ACIDS
3251175 ACID
          (ACID OR ACIDS)
     16 12S(W)HYDROXYEICOSATETRAENOIC(W)ACID
 909225 12
1765278 S
   1629 HYDROXYEICOSATETRAENOIC
2849710 ACID
1154220 ACIDS
3251175 ACID
          (ACID OR ACIDS)
     71 12(W)S(W)HYDROXYEICOSATETRAENOIC(W)ACID
 909225 12
   1942 HETE
    232 HETES
   1990 HETE
          (HETE OR HETES)
    823 12(W)HETE
   1185 12S
   1942 HETE
    232 HETES
   1990 HETE
          (HETE OR HETES)
     15 12S(W)HETE
 909225 12
1765278 S
   1942 HETE
    232 HETES
   1990 HETE
          (HETE OR HETES)
    146 12(W)S(W)HETE
      8 12HETE
      1 12SHETE
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3251175 ACID
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    434 12(W)HYDROXYEICOSATETRAENOIC(W)ACID
   1185 12S
   1629 HYDROXYEICOSATETRAENOIC
2849710 ACID
1154220 ACIDS
3251175 ACID
          (ACID OR ACIDS)
     16 12S(W)HYDROXYEICOSATETRAENOIC(W)ACID
 909225 12
1765278 S
   1629 HYDROXYEICOSATETRAENOIC
2849710 ACID
1154220 ACIDS
3251175 ACID
          (ACID OR ACIDS)
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        1942 HETE
         232 HETES
        1990 HETE
               (HETE OR HETES)
         823 12(W)HETE
        1185 12S
        1942 HETE
         232 HETES
        1990 HETE
               (HETE OR HETES)
          15 12S(W)HETE
      909225 12
     1765278 S
        1942 HETE
         232 HETES
        1990 HETE
               (HETE OR HETES)
         146 12(W)S(W)HETE
           8 12HETE
           1 12SHETE
L14     1138 L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9

=> s diagnos? or detect? or test? or assay? or marker or screen?
       88010 DIAGNOS?
      827475 DETECT?
     1130904 TEST?
      266110 ASSAY?
       44034 MARKER
       30576 MARKERS
       65514 MARKER
               (MARKER OR MARKERS)
      131638 SCREEN?
L15  2209040 DIAGNOS? OR DETECT? OR TEST? OR ASSAY? OR MARKER OR SCREEN?

=> s l14 and l15
L16      317 L14 AND L15

=> s a!t!er!osclero?
L17       20 A!T!ER!OSCLERO?

=> s atherosclero?
L18    18365 ATHEROSCLERO?

=> s artherosclero?
L19       54 ARTHEROSCLERO?

=> s arteriosclero?
L20     7815 ARTERIOSCLERO?

=> s arteriolosclero?
L21       17 ARTERIOLOSCLERO?

=> s atheriosclero?
L22       16 ATHERIOSCLERO?

=> s l17 or l18 or l19 or l20 or l21 or l22
L23    22115 L17 OR L18 OR L19 OR L20 OR L21 OR L22

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     FILE 'CA' ENTERED AT 13:08:19 ON 12 JUN 2003
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L1              QUE  ABB=ON  PLU=ON  12(W)HYDROXYEICOSATETRAENOIC(W)ACID
L2              QUE  ABB=ON  PLU=ON  12S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L3              QUE  ABB=ON  PLU=ON  12(W)S(W)HYDROXYEICOSATETRAENOIC(W)ACID
L4              QUE  ABB=ON  PLU=ON  12(W)HETE
L5              QUE  ABB=ON  PLU=ON  12S(W)HETE
L6              QUE  ABB=ON  PLU=ON  12(W)S(W)HETE
L7              QUE  ABB=ON  PLU=ON  12HETE
L8              QUE  ABB=ON  PLU=ON  12SHETE
L9              QUE  ABB=ON  PLU=ON  L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR
                ---------
L10         1475 S L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9
L11           20 S L10 AND P/DT
L12      3169683 S DIAGNOS? OR DETECT? OR TEST? OR ASSAY? OR MARKER OR SCREEN?
                SAVE TEMP L12 DIAGNOS/Q
L13            6 S L11 AND L12
                SET RANGE =(,13CI)
L14         1138 S L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9 RAN=(,13CI
L15      2209040 S DIAGNOS? OR DETECT? OR TEST? OR ASSAY? OR MARKER OR SCREEN? R
L16          317 S L14 AND L15 RAN=(,13CI)
L17           20 S A!T!ER!OSCLERO? RAN=(,13CI)
L18        18365 S ATHEROSCLERO? RAN=(,13CI)
L19           54 S ARTHEROSCLERO? RAN=(,13CI)
L20         7815 S ARTERIOSCLERO? RAN=(,13CI)
L21           17 S ARTERIOLOSCLERO? RAN=(,13CI)
L22           16 S ATHERIOSCLERO? RAN=(,13CI)
L23        22115 S L17 OR L18 OR L19 OR L20 OR L21 OR L22 RAN=(,13CI)
                SAVE TEMP L23 ATHERO/Q

=> s l16 and l23
L24           14 L16 AND L23

=> d l24 bib ab 1-14
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L24 ANSWER 1 OF 14 CA COPYRIGHT 2003 ACS

AN 124:146017 CA

TI Synthesis of 2(1H)-quinolinone derivatives and their inhibitory activity on the release of **12(S)-hydroxyeicosatetraenoic acid (12-HETE**) from platelets

AU Uno, Tetsuyuki; Ozeki, Yasushi; Koga, Yasuo; Chu, Gil-Namg; Okada, Minoru; Tamura, Katsumi; Igawa, Takehiro; Unemi, Fumiko; Kido, Masaru; Nishi, Takao

CS Second Inst. New Drug Res., Otsuka Pharmaceutical Co., Ltd., Tokushima, 771-01, Japan

SO Chemical & Pharmaceutical Bulletin (1995), 43(10), 1724-33
CODEN: CPBTAL; ISSN: 0009-2363

PB Pharmaceutical Society of Japan

DT Journal

LA English

AB A search for potent inhibitors of release of **12(S)-hydroxyeicosatetraenoic acid (12-HETE**), which plays an important role in the pathogenesis of various circulatory disorders and **arteriosclerosis**, led the authors to 6-[4-(1-cyclohexyl-5-tetrazolyl)butoxy]-3,4-dihydro-2(1H)-quinolinone (cilostazol) and 2(1H)-quinolinone derivs. having an azole group in the side chain. Many 2(1H)-quinolinone derivs. were synthesized and

tested in vitro for the inhibitory activity in human platelets. [(Tolylimidazolyl)sulfinylpropoxy]quinolinone I was one of the most potent inhibitors of **12-HETE** release, being more potent than esculetin. In addn., the sulfoxide I showed in vivo inhibitory activity on platelet adhesion in rats. Since I is racemic, the enantiomers were prepd. and their potencies were compared in vitro and in vivo. (S)-(+)-I had the best pharmacol. profile and was selected as a candidate drug for further development. The structure-activity relationships are discussed.

L24 ANSWER 2 OF 14 CA COPYRIGHT 2003 ACS
AN 124:83682 CA
TI Transgenic rabbits with the integrated human 15-lipoxygenase gene driven by a lysozyme promoter: macrophage-specific expression and variable positional specificity of the transgenic enzyme
AU Shen, Jianhe; Kuehn, Hartmut; Petho-Schramm, Attila; Chan, Lawrence
CS Dep. of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX, 77030, USA
SO FASEB Journal (1995), 9(15), 1623-31
CODEN: FAJOEC; ISSN: 0892-6638
PB Federation of American Societies for Experimental Biology
DT Journal
LA English
AB Lipoxygenase is expressed in foamy macrophages of **atherosclerotic** lesions and has been implicated in the oxidative modification of low-d. lipoprotein during early stages of atherogenesis. To establish an animal model of 15-lipoxygenase over-expression, the authors created transgenic rabbits that express at high level the 15-lipoxygenase in monocyte-derived macrophages but not in liver, heart, kidney, lung, or other tissue. The expression level of the enzyme in monocyte-derived macrophages is comparable to that of interleukin-4 (IL-4)-treated human monocytes, but more than 20-fold higher than that in macrophages of normal rabbits. The transgenic enzyme oxygenates linoleic acid to 13S-hydroperoxy-9,11 (Z,E)-octadecadienoic acid (13-HODE), and arachidonic acid to a mixt. of **12S-HETE** and 15S-HETE. The **12S-HETE** /15S-HETE ratio varied between 0.3 and 5.4, indicating a remarkable variability in the positional specificity of the transgenic enzyme. Macrophages from normal rabbits consistently produced **12S-HETE** as the major oxygenation product. The 15-lipoxygenase-overexpressing rabbits may be used for further mechanistic studies on the implication of lipoxygenase in atherogenesis; they are also an ideal model for **testing** the in vivo action of 15-lipoxygenase inhibitors.

L24 ANSWER 3 OF 14 CA COPYRIGHT 2003 ACS
AN 123:25427 CA
TI The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease
AU Pace-Asciak, Cecil R.; Hahn, Susan; Diamandis, Eleftherios P.; Soleas, George; Goldberg, David M.
CS Department of Pharmacology, University of Toronto and Research Institute, Hospital for Sick Children, Toronto, Can.
SO Clinica Chimica Acta (1995), 235(2), 207-19
CODEN: CCATAR; ISSN: 0009-8981
PB Elsevier
DT Journal
LA English
AB A no. of lines of evidence suggest that red wine may be more effective than other alc. beverages in decreasing the risk of coronary heart disease (CHD) mortality. This protection over and above that due to ethanol itself may be explained by phenolic components with which red wines are richly endowed. The authors have studied the effects of the trihydroxy stilbene trans-resveratrol on human platelet aggregation and on the synthesis of three eicosanoids from arachidonate by platelets, i.e. thromboxane B2 (TxB2), hydroxyheptadecatrienoate (HHT) and

12-hydroxyeicosatetraenoate (**12-HETE**). These effects were compared with the actions of other wine phenolics (quercetin, catechin and epicatechin) and antioxidants (.alpha.-tocopherol, hydroquinone and butylated hydroxytoluene). Trans-Resveratrol and quercetin demonstrated a dose-dependent inhibition of both thrombin-induced and ADP-induced platelet aggregation, whereas ethanol inhibited only thrombin-induced aggregation. The other compds. **tested** were inactive. Trans-resveratrol also inhibited the synthesis of TxB2, HHT, and to a lesser extent **12-HETE**, from arachidonate in a dose-dependent manner. Quercetin inhibited only **12-HETE** synthesis, and hydroquinone caused slight inhibition of TxB2 synthesis, the remaining compds. being ineffective. De-alcoholized red wines inhibited platelet aggregation; their ability to inhibit the synthesis of TxB2 but not that of **12-HETE** from labeled arachidonate by washed human platelets was proportional to their trans-resveratrol concn. These results are consistent with the notion that trans-resveratrol may contribute to the presumed protective role of red wine against **atherosclerosis** and CHD.

L24 ANSWER 4 OF 14 CA COPYRIGHT 2003 ACS
AN 122:154914 CA
TI Oxidative modification of human lipoproteins by lipoxygenases of different positional specificities
AU Kuehn, Hartmut; Belkner, Jutta; Suzuki, Hiroshi; Yamamoto, Shozo
CS Inst. Biochem., Univ. Clinicum Charite, Berlin, 10115, Germany
SO Journal of Lipid Research (1994), 35(10), 1749-59
CODEN: JLPRAW; ISSN: 0022-2275
PB Lipid Research, Inc.
DT Journal
LA English
AB Cellular lipoxygenases have been implicated in foam cell formation during the early stages of atherogenesis. The authors studied the interaction of lipoxygenases of different positional specificities with human lipoproteins and found that the arachidonate 15-lipoxygenases of rabbit and humans and the arachidonate 12-lipoxygenase of porcine leukocytes oxygenate lipoproteins as indicated by the formation of oxygenated lipids and changes in electrophoretic mobility of low d. lipoprotein. The arachidonate 12-lipoxygenase of human platelets, the recombinant arachidonate 5-lipoxygenase of human leukocyte, and the soybean lipoxygenase I were less effective in oxidizing human LDL. As a major oxygenation product, esterified 13S-hydro(pero)xy-9Z,11E-octadecadienoic acid was identified for both the rabbit reticulocyte 15- and the porcine leukocyte 12-lipoxygenase. In addn., esterified 15S-hydro(pero)xy-5,8,11,13(Z,Z,Z,E)-eicosatetraenoic acid (for the rabbit 15-lipoxygenase) and 12S-hydro(pero)xy-5,8,10,14(Z,Z,E,Z)-eicosatetraenoic acid (for the porcine 12-lipoxygenase) as well as small amts. of racemic 9-hydro(pero)xy-10,12-octadecadienoic acid isomers were **detected**. More than 90% of the oxygenated polyenoic fatty acids were found in the ester lipid fraction, particularly in the cholesteryl esters and in various phospholipid classes (phosphatidylcholine and phosphatidylethanolamine). The possible biol. significance of lipoxygenase-induced oxidative modification of lipoproteins in the pathogenesis of **atherosclerosis** is discussed.

L24 ANSWER 5 OF 14 CA COPYRIGHT 2003 ACS
AN 122:105410 CA
TI Preparation of caffeic acid amide derivatives as 12-lipoxygenase inhibitors
IN Matsuki, Shinsuke; Kiso, Yoshinobu; Cho, Hidetsura; Tamaoka, Mie; Murota, Seiitsu; Morita, Ikuo
PA Suntory Ltd, Japan
SO Jpn. Kokai Tokkyo Koho, 40 pp.
CODEN: JKXXAF
DT Patent

LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 06247850	A2	19940906	JP 1993-57991	19930224
PRAI	JP 1993-57991		19930224		

OS MARPAT 122:105410

AB Caffeic acid amide derivs. [I; R1, R2 = H, COR4, C(S)R5, PO(OR6)OR7, or R1R2 forms a 5-membered ring; wherein R4 = C1-6 alkyl or alkoxy, C6-10 aryloxy, c712 aralkyloxy, substituted amino, cyclic amino; R6, R7 = C1-6 alkyl, C6-10 aryl, C7-12 aralkyl, alkali metal; R3 = OR1, OR2, H, OH, O2CR4, OC(S)R5, PO(OR6)OR7, wherein R1, R2, R4 - R7 = same as above; X, Y = H, (un)substituted C1-6 alkyl, C6-10 aryl, C7-12 aralkyl, C7-12 aralkyloxy, C7-12 arylalkenyl, C7-12 aryloxyalkenyl, heterocyclyl, or heterocyclylalkyl, or XY forms N-contg. heterocyclic ring; provided that both X = Y .noteq. H] and pharmacol. acceptable salts thereof, useful for the treatment of **arteriosclerosis**, ischemic heart diseases, etc., are prepd. A medicament for the treatment and prevention of diseases caused by unusual rise in the activity of 12-lipoxygenase, e.g. atrophy of brain blood vessel, allergy, inflammation, cancer metastasis, asthma, normal psoriasis, and nephritis, contains 12-lipoxygenase inhibitor or pharmacol. acceptable salts thereof as the active ingredient. Thus, a soln. of 2.40 g 3,4-dihydroxybenzaldehyde in DMF was added to a soln. of N-[2-(2-thienyl)ethyl]-2-cyanoacetamide in DMF and benzene followed by adding a few drops of piperidine and the resulting mixt. was refluxed for 1 h to give 86% I (R1 = R2 = R3 = X = H, Y = Q) (II). In 12-lipoxygenase inhibition **assay**, II and I (R1 = R2 = R3 = X = H, Y = Q1) at 10-6 M in vitro inhibited the prodn. of **12-HETE** in rat platelet rich plasma,by 77.2 and 80.1%, resp.

L24 ANSWER 6 OF 14 CA

AN 121:227379 CA

TI Osteopontin-stimulated vascular smooth muscle cell migration is mediated by .beta.3 integrin

AU Yue, Tian-Li; Mckenna, Patrick J.; Ohlstein, Eliot H.; Farach-Carson, Mary C.; Butler, William T.; Johanson, Kyung; McDevitt, Patrick; Feuerstein, Giora Z.; Stadel, Jeffrey M.

CS Dep. Cardiovascular Pharmacology, Protein Biochemistry, SmithKline Beecham Pharmaceuticals, Pennsylvania, PA, 19406, USA

SO Experimental Cell Research (1994), 214(2), 459-64
CODEN: ECREAL; ISSN: 0014-4827

DT Journal

LA English

AB Osteopontin (OPN), a 41-kDa phosphorylated glycoprotein, has been **detected** in rat aorta and carotid arteries, and expression of its mRNA in blood vessels is strongly increased in response to vascular injury. To investigate the potential role of OPN in vascular pathophysiol., the authors studied the effect of rat OPN on aortic smooth muscle cell migration and proliferation in vitro. OPN enhanced the migration of rat smooth muscle cells in a time- and concn.-dependent manner with an EC50 value of 46 .+-. 11 nmol/L (n = 5). The maximal increase in cell migration by OPN was 29-fold over basal levels. OPN-induced smooth muscle cell migration was inhibited in a concn.-dependent manner by the monoclonal antibody F11, which recognizes the rat integrin subunit .beta.3; in contrast, polyclonal antiserum recognizing the rat integrin .beta.1 subunit did not inhibit smooth muscle cell migration in response to OPN, but did block fibronectin-promoted migration. Moreover, OPN-induced smooth muscle cell migration was dependent on the presence of extracellular divalent cations and was significantly inhibited by anti-OPN antibodies. OPN did not stimulate [3H]thymidine incorporation into cultured smooth muscle cells, indicating that it selectively enhanced migration. In view of the pathol. significance of arterial smooth muscle cell migration in the formation of intimal thickening, the results suggest that smooth muscle cell

recognition of OPN, probably through the vitronectin receptor, .alpha.v.beta.3, could play a role in the cells' response to vascular injury and esp. neointima formation.

L24 ANSWER 7 OF 14 CA COPYRIGHT 2003 ACS
AN 117:108868 CA
TI Effects of monohydroxylated fatty acids on arterial smooth muscle cell properties
AU Ramboer, Isabelle; Blin, Patrice; Lacape, Genevieve; Daret, Daniele; Lamaziere, Jean Marie Daniel; Larrue, Jacky
CS Cardiol. Res. Unit, INSERM U8, Pessac, Fr.
SO Kidney International, Supplement (1992), 41(37), 67-72
CODEN: KISUDF; ISSN: 0098-6577
DT Journal
LA English
AB The hydroxylated derivs. of polyunsatd. fatty acids may be potent modulators of basic biol. responses involved in pathol. processes, including **atherosclerosis**. The object of the present investigation was to study the effects of monohydroxylated fatty acids (namely **12-hydroxyeicosatetraenoic acids, 12-HETE**) on the properties of aortic smooth muscle cells (SMC) in culture. The changes in cell expression of differentiation antigen .alpha.-SM actin and 2P1A2 were followed by computerized morphometry, using specific monoclonal antibodies and the activation of cells by measuring cell motility. In addn., intracellular [Ca2+]i mobilization and inositol trisphosphate (IP3) formation were studied. Finally, the metabolic routes of monohydroxylated compds. and their effects on PGI2 secretion were reported. The results demonstrate that **12-HETE** is able to stimulate the phenotypic modulation, PGI2 prodn. and motility of arterial SMCs, despite any **detectable** activity in increasing [Ca2+]i or IP3 formation. By contrast with parent compds. 15-HETE and 13-HODE, which appear as potent prodifferentiating mols., **12-HETE** is specifically metabolized via a 10-11 reductase pathway in addn. to the classical .beta.-oxidn. pathway. Taken together, these results suggest that cellular metab. of **12-HETE**, produced by platelets in the vicinity of the arterial intima, and also by cells present inside the **atherosclerotic** intima, or assocd. with modified LDL may play a key role in the **atherosclerotic** process.

L24 ANSWER 8 OF 14 CA COPYRIGHT 2003 ACS
AN 115:229466 CA
TI Autocrine system for smooth muscle cell migration and proliferation in the arterial wall
AU Saito, Yasushi; Morisaki, Nobuhiro; Koyama, Noriyuki
CS 2nd Dep. Intern. Med., Chiba Univ., Chiba, 280, Japan
SO Annals of the New York Academy of Sciences (1990), 598(Atherosclerosis 2), 194-9
CODEN: ANYAA9; ISSN: 0077-8923
DT Journal
LA English
AB The CM of cultured SMC from rat and rabbit aorta at passage 2 to 12 was **tested** for the presence of a factor that induced migration of SMC. The activity was compared with that of purified PDGF. Results showed that cultured SMC secreted a factor with strong activity to induce migration of SMC. The authors named this factor smooth muscle-derived migration factor (SDMF). The results of physicochem. analyses showed that SDMF is a new migration factor, distinct from various factors reported previously, such as PDGF, interleukin 1, fibronectin, leukotriene B4, and **12-HETE**. This conclusion was supported by immunol. studies with anti-PDGF and anti-fibronectin antisera. The migration activity of the CM cultured intimal SMC from the thickened intima of rabbits given high cholesterol diet was about twice that of CM of medial SMC. Thus in atheromatous lesions, SDMF may enhance the migration of the medial SMC to

the intima, resulting in a vicious cycle of migration and proliferation of SMC. This autocrine system inducing migration of SMC may contribute to the development of atheromatous lesions.

L24 ANSWER 9 OF 14 CA COPYRIGHT 2003 ACS
AN 114:183145 CA
TI The oxygenation of cholesterol esters by the reticulocyte lipoxygenase
AU Belkner, Jutta; Wiesner, Rainer; Kuehn, Hartmut; Lankin, V. Z.
CS Sch. Med., Humboldt Univ., Berlin, O-1040, Germany
SO FEBS Letters (1991), 279(1), 110-14
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English
AB The arachidonate 15-lipoxygenase from rabbit reticulocytes oxygenates cholesterol esters contg. polyenoic fatty acids. Cholesterol esterified with satd. fatty acids is not oxygenated. The structures of the oxygenation products formed from various cholesterol esters have been identified by high pressure liq. chromatog., UV-spectroscopy and gas chromatog./mass spectroscopy. Oxygenated cholesterol esters have been **detected** in **atherosclerotic** plaques of human aortas.

L24 ANSWER 10 OF 14 CA COPYRIGHT 2003 ACS
AN 112:197871 CA
TI Preparation and formulation of caffeic acid derivatives as 12-lipoxygenase inhibitors
IN Cho, Hidetsura; Ueda, Masaru; Tamaoka, Mie; Hamaguchi, Mikiko; Murota, Seiitsu; Morita, Ikuo
PA Suntory, Ltd., Japan
SO Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	EP 339671	A2	19891102	EP 1989-107771	19890428
	EP 339671	A3	19910515		
	EP 339671	B1	19940810		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 01275552	A2	19891106	JP 1988-106274	19880428
	JP 2557090	B2	19961127		
	JP 02235852	A2	19900918	JP 1989-55867	19890308
	JP 2647185	B2	19970827		
	AU 8933813	A1	19891102	AU 1989-33813	19890428
	AU 618595	B2	19920102		
	US 5063243	A	19911105	US 1989-344583	19890428
PRAI	JP 1988-106274		19880428		
	JP 1989-55867		19890308		

OS MARPAT 112:197871
AB Caffeic acid derivs. [I; X = H, OH; R1 = H, straight or branched C1-20 alkyl or alkenyl, (CH2)nZR2; n = 1-10; Z = O, CH:CH, a single bond; R2 = (un)substituted Ph or heterocyclyl], useful for curing and preventing circulatory diseases, e.g. **arteriosclerosis**, are prepd. Thus, to a soln. of 3,4-dihydroxybenzaldehyde were added NCCH2CO2Et and piperidine and the resulting mixt. was kept 20 h at room temp. to give I (X = H, R1 = Et) (II). In 12-lipoxygenase inhibition **test**, II lowered the prodn. of **12-hydroxyeicosatetraenoic acid** (5-HETE) by 38.1% in a dild. and citrated blood collected from the abdominal aorta of rat.

L24 ANSWER 11 OF 14 CA COPYRIGHT 2003 ACS
AN 110:190259 CA
TI Formation of 15-hydroxyeicosatetraenoic acid (15-HETE) as the predominant eicosanoid in aortas from Watanabe Heritable Hyperlipidemic and

cholesterol-fed rabbits
AU Simon, Theodore C.; Makheja, Amar N.; Bailey, J. Martyn
CS Sch. Med., George Washington Univ., Washington, DC, 20037, USA
SO Atherosclerosis (Shannon, Ireland) (1989), 75(1), 31-8
CODEN: ATHSBL; ISSN: 0021-9150
DT Journal
LA English
AB **Atherosclerotic** plaque formation is accompanied by hyperproliferative events which have many features of an inflammatory response. A HPLC procedure was developed to analyze the inflammatory prostaglandins, leukotrienes, and hydroxyeicosatetraenoic acids (HETEs) produced by aortic segments. Normal rabbit aortas incubated with [3H]arachidonic acid synthesized **12-HETE** as the principal lipoxygenase metabolite and prostacyclin as the major cyclooxygenase product. In contrast, **atherosclerotic** aortas from both cholesterol-fed and Watanabe Heritable Hyperlipidemic rabbits (**atherosclerotic** plaque formation models) showed major increases in synthesis of lipoxygenase-derived 15-HETE, which became the predominant eicosanoid in the aortas of both types of rabbits. No leukotrienes or other 5-lipoxygenase products were **detected**. 15-HETE, which is chemotactic for smooth muscle cells, mitogenic for endothelial cells, and an inhibitor of prostacyclin synthesis may thus play a role in atherogenesis.

L24 ANSWER 12 OF 14 CA COPYRIGHT 2003 ACS
AN 104:204966 CA
TI Platelet formation of **12-hydroxyeicosatetraenoic acid** and thromboxane B2 is increased in type IIA hypercholesterolemic subjects
AU Eynard, A. R.; Tremoli, E.; Caruso, D.; Magni, F.; Sirtori, C. R.; Galli, G.
CS Inst. Pharmacol. Pharmacogn., Univ. Milan, Milan, Italy
SO Atherosclerosis (Shannon, Ireland) (1986), 60(1), 61-6
CODEN: ATHSBL; ISSN: 0021-9150
DT Journal
LA English
AB The formation of the major metabolic products of endogenous arachidonic acid (AA) via cyclooxygenase and lipoxygenase pathways in platelets from normal and type IIA hypercholesterolemic subjects was evaluated. **12-Hydroxyeicosatetraenoic acid** (**12-HETE**) and TXB2 were detd. by selective ion monitoring (SIM) after extn. and purifn. of collagen stimulated platelet-rich plasma (PRP). The levels of both arachidonic acid metabolites in the nonstimulated PRP of control and type IIA subjects were below the **detection** limit of the method, but after collagen stimulation both metabolites were **detected**. Both **12-HETE** and TXB2 levels in collagen-stimulated PRP samples from the patients were higher than levels in controls. In view of the key role of **12-HETE** in mediating smooth muscle cell migration and proliferation and in stimulating macrophage activity, these data may provide information for the understanding of the elevated incidence of thrombosis and atheromatous lesion in patients with type IIA hypercholesterolemia.

L24 ANSWER 13 OF 14 CA COPYRIGHT 2003 ACS
AN 102:18388 CA
TI Eicosanoid effects on cell proliferation in vitro: relevance to **atherosclerosis**
AU Smith, Donald L.; Willis, Anthony L.; Mahmud, Ishtiaq
CS Inst. Pharmacol. Metab., Syntex Res., Palo Alto, CA, 94304, USA
SO Prostaglandins, Leukotrienes and Medicine (1984), 16(1), 1-10
CODEN: PLMEDD; ISSN: 0262-1746
DT Journal
LA English
AB Several eicosanoids were **tested** for ability to inhibit

proliferation of cells in culture. In rabbit aortic smooth muscle cells and mouse B6BL6 melanoma cells the potency order was: **12-hydroxyeicosatetraenoic acid** [71030-37-0] > PGJ2 [60203-57-8], > PGA1 [14152-28-4] .gtoreq. PGE1 [745-65-3] > PGE2 [363-24-6] .gtoreq. PGD2 [41598-07-6] .gtoreq. PGA2 [13345-50-1]. PGB1 [13345-51-2] was active in smooth muscle cells (GPGO2) but not in B16 cells. 5-Hydroxyeicosatetraenoic acid [71030-39-2] and LTB4 [71160-24-2] were weakly active in smooth muscle cells, and PGB2, PGF2.alpha., and TXB2 were inactive in both cells types. In Swiss albino mouse 3T3 fibroblasts, PGJ2 and PGE1 showed much lower relative potency than in the other 2 cell lines, although the profile was otherwise similar. These findings may be relevant to the antiatherosclerotic (and perhaps antitumor activity) of some eicosanoids.

L24 ANSWER 14 OF 14 CA
AN 97:196385 CA
TI Comparative effect of lipoxygenase products of arachidonic acid on rat aortic smooth muscle cell migration
AU Nakao, Junko; Ooyama, Toshiro; Ito, Hideki; Chang, Wen Chang; Murota, Seiitsu
CS Dep. Intern. Med., Tokyo Metrop. Geriatr. Hosp., Tokyo, 173, Japan
SO Atherosclerosis (Shannon, Ireland) (1982), 44(3), 339-42
CODEN: ATHSBL; ISSN: 0021-9150
DT Journal
LA English
AB The effects of monohydroxyeicosatetraenoic acids (HETEs) and F-Met-Leu-Phe (where F = formyl) on rat aortic smooth muscle cell migration were studied in modified Boyden chambers. **12-HETE** showed the most potent stimulatory effect on smooth muscle cell migration among the HETEs **tested**. The optimal concns. for cell migration were 3 .times. 10-15 and 3 .times. 10-13 g/mL for **12-HETE** and 10-8 g/mL for 15-HETE. 5-HETE and F-Met-Leu-Phe were inactive with these cells. As **12-HETE** is biosynthesized from arachidonic acid by the 12-lipoxygenase pathway in platelets and macrophages, and 15-HETE by the 15-lipoxygenase pathway in granulocytes, the present results indicate an important role for such cells in the early phase of **atherosclerosis**.

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